# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*



**PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ORGANIZATION.**

| 1. REPORT DATE *(DD-MM-YYYY)* | 2. REPORT TYPE | 3. DATES COVERED *(From - To)* |
|---|---|---|
| 25-07-2007 | Final Performance Report | 01-05-2005 to 30-04-2007 |

**4. TITLE AND SUBTITLE**
Research on Non-lethal Stunning/Immobilizing Weapons

**5a. CONTRACT NUMBER**

**5b. GRANT NUMBER**
FA9550-05-1-0308

**5c. PROGRAM ELEMENT NUMBER**
61102F

**6. AUTHOR(S)**
Craviso, Gale L.
Chatterjee, Indira

**5d. PROJECT NUMBER**
2301/EX

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**
University of Nevada, Reno
Sponsored Projects, Mail Stop 325
204 Ross Hall
Reno, NV 89557-0240

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
USAF/AFRL
Air Force Office of Scientific Research
875 North Randolph Street, Room 3112
Arlington, VA 22203
Dr Robert Barker/NE

AFRL-SR-AR-TR-07-0292

**12. DISTRIBUTION/AVAILABILITY STATEMENT**
Unlimited
Distribution Statement A: Unlimited

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**

The overall goal of the research is to lay the foundation for developing non-lethal stunning/immobilizing weaponry based on radiofrequency (RF)/microwave(MW) radiation. Our approach is to identify RF/MW parameters that can selectively, and without producing heating of tissue, alter processes underlying neurotransmitter release and contraction of skeletal muscle. Major accomplishments included 1) completing the design, construction, characterization and testing of a temperature control setup for studying effects of rapid increases in temperature so that we can distinguish thermal versus non-thermal effects of the exposures; 2) completing thermal modeling of the cell perfusion system used for on-line monitoring of catecholamine release from chromaffin cells during RF/MW exposure; and 3) designing, fabricating and characterizing an exposure system for real-time imaging of intracellular effects on chromaffin cells and skeletal muscle fibers in response to high electric field RF/MW pulse modulated radiation. The research has been presented at four international meetings, culminated in two peer-reviewed papers, and involved a neurobiologist and an electrical engineer as principal investigators, an associate engineer, four research assistants and four graduate students.

**15. SUBJECT TERMS**
Radiofrequency/microwave fields, non-thermal bioeffects, chromaffin cells, catecholamine release, skeletal muscle contraction, on-line and real-time monitoring of bioeffects

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
|---|---|---|---|---|---|
| a. REPORT | b. ABSTRACT | c. THIS PAGE | | | Gale L. Craviso |
| Unclassified | Unclassified | Unclassified | None | 12 | 19b. TELEPHONE NUMBER *(Include area code)* 775-784-4118 |

# FINAL PERFORMANCE REPORT

**Technical Proposal entitled:** "Research on non-lethal stunning/immobilizing weapons"

**Award Number:** FA9550-05-1-0308

**Start Date:** 01 May 2005

**Termination Date:** 30 April 2006; no cost extension until 30 April 2007

**Principal Investigator:** Gale L. Craviso, Ph.D.
Professor of Pharmacology
Dept. of Pharmacology
Howard Building, Room 219
University of Nevada School of Medicine
Reno, NV 89557
Phone: 775-784-4118
Fax: 775-784-1620
Email: gcraviso@medicine.nevada.edu

## ABSTRACT

The overall goal of the research is to lay the foundation for developing non-lethal stunning/immobilizing weaponry based on radiofrequency (RF)/microwave(MW) radiation. Our approach is to identify RF/MW parameters that can selectively, and without producing heating of tissue, alter processes underlying neurotransmitter release and contraction of skeletal muscle. Major accomplishments included 1) completing the design, construction, characterization and testing of a temperature control setup for studying effects of rapid increases in temperature so that we can distinguish thermal versus non-thermal effects of the exposures; 2) completing thermal modeling of the cell perfusion system used for on-line monitoring of catecholamine release from chromaffin cells during RF/MW exposure; and 3) designing, fabricating and characterizing an exposure system for real-time imaging of intracellular effects on chromaffin cells and skeletal muscle fibers in response to high electric field RF/MW pulse modulated radiation. The research has been presented at four international meetings, culminated in two peer-reviewed papers and one Master's thesis, and involved a neurobiologist and an electrical engineer as principal investigators, an associate engineer, four research assistants and four graduate students. The research has been transitioned into AFOSR grant FA9550-06-1-0377.

## EXECUTIVE SUMMARY

### Objectives

The research in this proposal was to sustain the progress and growth of on-going research projects in which non-thermal radiofrequency /microwave (RF/MW) effects on skeletal muscle contraction and catecholamine release from chromaffin cells are being investigated. With respect to the latter effort, one objective was to use single cell amperometry to measure in real-time the influence of rapid incremental increases in temperature above 36° - 37°C on the rate and amount of catecholamines released both spontaneously and in response to a stimulus. Although our experimental approach includes several measures to ensure that changes in catecholamine release observed during RF/MW exposure are not related to increases in temperature, an understanding of the basic effect of heating on catecholamine release also was needed for providing convincing evidence of effects that are likely the result of a direct non-thermal interaction of RF/MW radiation on the secretory process. A second objective was to carry out thermal modeling of our cell perfusion apparatus used for on-line monitoring of catecholamine release so that we can also quantify the temperature distribution where the cells are located during exposures.

In order to explore further ways of eliciting non-thermal bioeffects via RF/MW radiation, a third objective encompassing studies examining effects on both catecholamine release and skeletal contraction was to develop novel RF/MW nanopulsing technologies for inclusion in our RF/MW exposure protocols.

### Accomplishments/New Findings

- The design, construction, characterization and testing of a temperature control setup for monitoring catecholamine release from chromaffin cells via amperometry in response to rapid increases in temperature has been completed and tested.
- Transient thermal/flow modeling has been carried out for the cell perfusion apparatus used in our setups in which catecholamine release from perfused chromaffin cells is continuously monitored on-line during RF/MW exposure of the cells.
- A novel exposure system for real-time imaging of intracellular effects in response to high electric field RF/MW pulse modulated radiation, broadband Gaussian pulses or RF/MW modulated Gaussian pulses with frequency spectrum centered in the band 0.75–6 GHz has been designed, fabricated and characterized. Testing is underway.
- A collaboration that has been established with researchers at USC to explore effects of nsec duration, high intensity electric field effects on chromaffin cells has been very productive.

### Publications

Yoon, J., Chatterjee, I., D. M., Craviso, G. L. (2006). Design, characterization and optimization of a broadband mini exposure chamber for studying catecholamine release from chromaffin cells exposed to microwave radiation: Finite-Difference Time-Domain Technique. IEEE Transactions on Plasma Science, 34, 1455-1469.

Lambrecht, M., Chatterjee, I., McPherson, D., Quinn, J., Hagan, T., Craviso, G. L. (2006). Design, characterization and optimization of a waveguide-based RF/MW exposure system for studying nonthermal effects on skeletal muscle contraction. IEEE Transactions on Plasma Science, 34, 1470-1479.

**Thesis**

Bindya Dumpala completed her M.S. in Biomedical Engineering at the University of Nevada, Reno in December 2006; her thesis is titled "Design, Construction and Characterization of a Temperature Control System for Studying the Effects of Rapid and Reversible Changes in Temperature on Neurosecretion - Characterization and Optimization".

**Personnel Supported**

Gale L. Craviso, Ph.D., Professor of Pharmacology – Principal Investigator
Indira Chatterjee, Ph.D., Professor of Electrical and Biomedical Engineering – Co-Principal Investigator
Dana McPherson, Associate Engineer, Dept. of Electrical and Biomedical Engineering
Horace Goff, laboratory assistant
Robert Wiese, laboratory assistant
Sophie Choe, part-time laboratory assistant
Gabriel Maalouf, part-time laboratory assistant
Sue Chung, undergraduate laboratory aide
Bindya Dumpala, M.S. student, Biomedical Engineering
Todd Hagan, Ph.D. student in Electrical Engineering
Jihwan Yoon, Ph.D. student in Electrical Engineering
Paulo Vandenberg, M.S. student in Electrical Engineering
Ranjan Misra, electrical engineering undergraduate student from UCLA doing a summer research rotation

**Interactions/Transitions**

a) Presentations

Oral:

Lambrecht, M., Chatterjee, I., Quinn, J., McPherson, D. and Craviso, G.L. Design and optimization of a radiofrequency/microwave exposure system for assessing effects on skeletal muscle contraction 4th International Symposium on Nonthermal Medical/Biological Treatments Using Electromagnetic Fields and Ionized Gases, 2005.

Yoon, J., Chatterjee, I., McPherson, D. and Craviso, G.L. Design and characterization of a broadband mini exposure chamber for studying catecholamine release from chromaffin cells due to non-thermal levels of 1 – 6 GHz continuous and pulsed microwave radiation - Finite-Difference Time-Domain computations. 4th International Symposium on

Nonthermal Medical/Biological Treatments Using Electromagnetic Fields and Ionized Gases, 2005.

Hagan, T., Chatterjee, I., McPherson, D. and Craviso, G.L., Design and finite-difference time-domain characterization of a novel in vitro exposure device for real-time monitoring of changes in intracellular calcium due to pulsed RF/microwave electric fields. 28th Annual Bioelectromagnetics Society Meeting, Cancun, Mexico, June 2006.

Craviso, G.L., Sun, Y., Chen, M-T, Gundersen, M.A. and Vernier, P.T. Single Nanosecond Electric Pulse Elevates Intracellular Calcium in Bovine Adrenal Chromaffin Cells. 28th Annual Bioelectromagnetics Society Meeting, Cancun, Mexico, June 2006.

Craviso, G. L., Sun, Y., Chen, M., Gundersen, M. and Vernier, T., "Elevation of intracellular calcium in bovine adrenal chromaffin cells subjected to a single nanosecond electric pulse.", Gordon Conference on Bioelectrochemistry, Aussois, France. (September 2006).

Poster:

Lambrecht, M., Chatterjee, I., McPherson, D., Quinn, J. and Craviso, G.L. Finite-difference time-domain computations of SAR distribution within an exposure system for studying the effects of radiofrequency/microwave fields on skeletal muscle contraction. Joint Meeting of the Bioelectromagnetics Society and the European BioElectromagnetics Association, 2005.

Craviso, G. L., Brouse, D., Hagan, T., McPherson, D. and Chatterjee, I. Use of cultured adrenal chromaffin cells as an *in vitro* model system to study non-thermal effects of RF radiation on exocytosis. Joint Meeting of the Bioelectromagnetics Society and the European BioElectromagnetics Association, 2005.

Other:

The research was mentioned in the December 2006 issue of Popular Mechanics

b) Consultative and advisory functions – None

c) Transitions – When funding for this grant ends, the research project will transition into AFOSR FA9550-06-1-0377.

**New Discoveries, Inventions or patent disclosures:** We are in the process of applying for a patent for the temperature control system that we have designed and fabricated.

**Honors/Awards** - None

## COMPREHENSIVE TECHNICAL SUMMARY

### Rationale

Since a plausible way to disrupt human functioning is by altering neuronal activity and skeletal muscle contraction, the research to investigate the feasibility of designing new non-lethal weapons based on RF/MW radiation is focused on determining how RF/MW radiation can be applied to produce non-thermal effects on neurotransmitter release and contractile activity, respectively. The rationale behind the research is that biomolecules, specifically plasma membrane proteins, are believed to undergo non-thermal alterations in function in response to RF/MW radiation.

To carry out these studies, an important objective has been to design, fabricate, characterize and test RF/MW exposure systems that allow maximal flexibility in choosing exposure parameters (e.g., frequency, modulation schemes, etc.) as well as permit optimal handling of samples under the constraints and limitations imposed by the biological experiment.

### Specific Projects and Outcomes

#### 1) Studying the influence of rapid temperature increases on catecholamine release from chromaffin cells.

Important to the successful identification of non-thermal RF/MW bioeffects is a detailed investigation of how rapid changes in temperatures in excess of the normal physiologic temperature range of 36-38°C affect the bioresponse being measured (in our case, catecholamine release that is measured using single cell amperometry). Because a temperature control device that can provide the rapid, controlled and reversible changes in temperature that this type of study requires is neither available commercially nor described in the literature, we undertook the design, fabrication and testing of one that would meet our needs. All details are given in the Master's thesis of Bindya Dumpala.

Important features of the system is that it is capable of eliciting an average rate of change in temperature (both increases and decreases) of 30°C per second at the location where chromaffin cells are immobilized on the bottom of a culture dish (Appendix, Figure 1). Dynamic temperature control is achieved by a temperature control feedback loop that provides a fully automated temperature control system. This feature is critical for reproducibility of experimental conditions. Figure 2 (Appendix) shows that the presence of chromaffin cells attached to the bottom of the culture dish does not affect the performance of the temperature control apparatus.

Throughout the design process, the drawing software Solid Works, used in conjunction with the thermal modeling software Cosmos Works, provided us with an assessment of the temperature transients produced where the cells are attached as well as in the balanced salt solution that surrounds the cells in the culture dish. The use of this software also allowed us to determine how to achieve the greatest homogeneity in the spatial distribution of temperature on

the bottom of the culture dish in the shortest period of time. This has resulted in the design of a second temperature control device that will be fabricated and tested in the future.

While we are currently exploring whether these systems can be patented, overall progress in conducting experiments using this heating device has been slow due to problems with the Peltier elements (PEs) that comprise the system. Although we purchased PEs that are potted (coated), repeated placement of the PEs in the balanced salt solution that bathes the cells during experiments leads to their eventual failure due to liquid seeping into the PEs. The manufacturer (there is only one that fabricates PEs with our specifications) has been unwilling to fabricate the PEs with a more resilient, biologically-compatible coating to avoid such failure so we are exploring how we can coat the PEs ourselves without negatively affecting their functioning. We are also looking into the design of a different PE arrangement so that the PEs would not be in direct contact with balanced salt solution.

Another setback has been the loss of personnel. The graduate student who took over the project from the one who graduated, decided, for personal reasons, to leave graduate school. Consequently, the project has been on hold until we can recruit another student.

**2) Thermal modeling**

In the exposure system we designed for on-line monitoring of catecholamine release from chromaffin cells exposed to microwave fields in the frequency range 1 – 6 GHz (Yoon, et al., 2006), average SAR values computed using the Finite-Difference Time-Domain method ranged from 0.05 – 67 W/kg over the region where cells would be located during the exposures. Moreover, the SAR distribution over this region had a certain degree of inhomogeneity. We therefore felt that it would be important to compute the resulting temperature distribution over this region by solving the heat conduction equation and accounting for the flow of the balanced salt solution (BSS) across the surface of and through the glass fiber filter where the cells are immobilized.

The exposure system consists of a cell perfusion apparatus (CPA) inside which chromaffin cells are immobilized on a glass fiber filter of diameter 24 mm. The cells are continuously superfused with temperature-controlled BSS at a rate of 0.4 ml/min. The temperature of the BSS entering and exiting the CPA is continuously monitored in the inlet and outlet tubing with non-perturbing fluoroptic temperature probes placed as close as physically possible to the glass fiber filter where the cells are immobilized. This is the best we can do since it is not possible to measure the temperature distribution at the exact location of the cells on the glass fiber filter. The CPA is mounted vertically within a mini anechoic chamber and the cells exposed to continuous wave microwave fields in the frequency range 1 - 6 GHz by positioning the CPA in the far field of a high power broadband horn antenna.

As described in Yoon et al. (2006), a commercially available Finite-Difference Time-Domain (FDTD) software package XFDTD (Remcom, Inc.) was used to compute the detailed SAR distribution over the glass fiber filter where the cells are immobilized. Over the frequency range 1 - 6 GHz, the average SAR varies from 0.05 to 67.25 W/kg, with the maximum SAR obtained at

around 3.5 GHz. This latter result is expected since at approximately 3.5 GHz, the diameter of the glass fiber filter is approximately half a wavelength in the glass fiber filter soaked with BSS and thus, coupling of the electric field would be maximal. The homogeneity of the SAR expressed as the percentage of area of the glass fiber filter over which the SAR is homogeneous to within 30% varies from 47% at 1 GHz to 18.7 % at 6 GHz.

Since the goal of the on-line experiments is to study non-thermal effects on catecholamine release, it is important to ensure that the temperature of the cells immobilized on the glass fiber filter is maintained to within an acceptable limit, i.e. 36 ± 0.2°C. Hence a detailed thermal model of the entire CPA was created (Appendix, Figure 3) and analyzed using the commercially available software package COSMOSFloWorks (SolidWorks Corp.). This model takes into account heat transfer by conduction and losses by radiation, inlet flow rate of the BSS (measured using a flow meter), and pressure at the outlet of the glass fiber filter (measured using a pressure transducer). The temperature of the solid at the boundaries of the model was set to room temperature (default of 20°C). Both inlet and outlet temperature (measured) were given as inputs to the model. The SAR distribution computed using XFDTD was input into the model as a heat source. The total analysis time was 3600 s with a time step of 60s.

The results of the thermal modeling (Appendix, Figure 4) indicated that without any SAR input, there was a large temperature gradient between the center and edge of the glass fiber filter. The temperature at the center was 36.1°C and that at the edge of the glass fiber filter was 20°C (i.e. room temperature). This alerted us to the possibility that perhaps the BSS was not diffusing fast enough across and through the porous glass fiber filter to maintain a uniform temperature distribution on the glass fiber filter, although the actual computed temperature difference appeared too high. After directly measuring the temperature at these locations on the glass fiber filter in the experimental set-up, a temperature gradient was indeed observed, but it was not as severe as that computed. At the center of the glass fiber filter the measured temperature was 36°C and at the edge of the glass fiber filter the measured temperature was 30.2°C. Hence, we are in the process of not only modifying our CPA design to decrease this temperature gradient (Yoon, et al., 2007), but we are also incorporating a better representation of the glass fiber filter into the thermal model so that it will predict more accurately the temperature distribution over the glass fiber filter. In addition, when the SAR distribution computed using XFDTD at 3.5 GHz, for an input power of 250W to the horn antenna, was supplied as a heat source to the thermal model, the temperature distribution obtained over the glass fiber filter was almost identical to that obtained without the SAR. This finding is very important as it indicates that the SAR levels being used in the experiments are non-thermal.

As stated above, the simulations show that as cells are being superfused, a temperature variation exists across the current glass fiber filter with diameter of 24 mm due to (1) heat transferred from the BSS to the CPA via conduction and, (2) the tendency of the BSS to flow faster through the central region of the glass fiber filter. To overcome these problems, the CPA has been replaced with a smaller one that incorporates a glass fiber filter of diameter 10 mm, a 5 μm nylon mesh of diameter 10 mm with a 5 mm hole in the middle is placed directly on top of the glass fiber filter to immobilize the cells within the central part of the glass fiber filter. The smaller CPA resulted in a lower measured temperature difference between the center and the edge of the glass fiber filter (from 5.8°C to 2.5°C) as well as between the inlet and outlet BSS

(from 4 °C to 1°C). Thus, the results of the thermal modeling allowed us to make some important design modifications of the CPA and the exposure system.

**3) Developing novel RF/MW nanopulsing technologies for real-time monitoring of RF/MW bioeffects.**

The waveguide-based exposure system we have been using to monitor effects on catecholamine release during RF/MW exposure of chromaffin cells has revealed modest but distinct changes in catecholamine release in the frequency range 750 - 850 MHz. Because the observed effects are not always reproducible, we felt that we were near a threshold with respect to electric field intensity and would thus need an exposure system that will be capable of producing higher E-field strengths than the waveguide system is capable of, without having to expend funds on another high power amplifier. We also wanted a system with greater sensitivity for measuring chromaffin cell RF/MW-induced bioeffects so that we could work at the lowest electric field intensity possible, provide assessment of RF/MW bioeffects in real-time (which would be an improvement over on-line measurements), and retain the ability to work at physiologic temperature. Finally, since the effects we have observed for catecholamine release have been over a band of frequencies (0.75 to 0.85 GHz), the system desired should be capable of exposing the cells to broadband fields in the range of frequencies 0.75 - 6 GHz, so as to allow us to identify narrower frequency bands within this larger frequency band where effects may be more pronounced. This project is well underway in our laboratory as we have designed and fabricated a unique exposure/experimental setup (Appendix, Figure 5) for carrying out real-time fluorescence imaging of intracellular calcium level and membrane potential in chromaffin cells. The system, which has been designed for use with an inverted microscope, can deliver to the cells high electric field (Appendix, Figure 6) RF/MW pulse modulated radiation, broadband Gaussian pulses or RF/MW modulated Gaussian pulses with frequency spectrum centered in the band 0.75 - 6 GHz. A photograph of the entire setup mounted on the inverted microscope is shown in Figure 7 (Appendix).

We are in the final stages of working out the logistically problems associated with this setup, specifically, vibrations due both to the perfusion of the cells and the coolant system used to prevent heating during RF/MW exposures. This involves machining new parts to improve the stability of the overall setup during cell imaging. Nevertheless, we anticipate beginning experiments within the next month. A post-doctoral fellow who joined our group last Fall is already very proficient not only in imaging chromaffin cells loaded with a variety of fluorescent dyes but also in operating the exposure system for conducting actual experiments.

**Ongoing work/future directions.** As stated in the annual Progress Report for last year, we have initiated a collaboration with Tom Vernier and Martin Gundersen at the University of Southern California (USC) to look at the effect of nanosecond high intensity electric pulses on intracellular calcium level in chromaffin cells. We determined that this was the fastest way to explore how chromaffin cells, which are electrically excitable, respond to nanosecond pulsed electric fields. We found that intracellular calcium increases immediately upon the application of a single 4 nanosecond pulse (Appendix, Figure 8) and these results were presented at two international meetings; a manuscript describing these results is in preparation. We will be

continuing the collaboration with Drs. Vernier and Gundersen by working out the conditions to determine whether the single burst of calcium is sufficient to be of physiological significance, that is, trigger catecholamine release.

Our main goal is to continue experiments that utilize the RF/MW exposure systems designed and optimized by our group. For the free-space exposure experiments, we still need to optimize conditions for working at each frequency using the smaller CPA. This will be done as experiments begin, which we anticipate will be very soon.

To continue all our efforts, the research has been transitioned into AFOSR grant FA9550-06-1-0377.

## APPENDIX

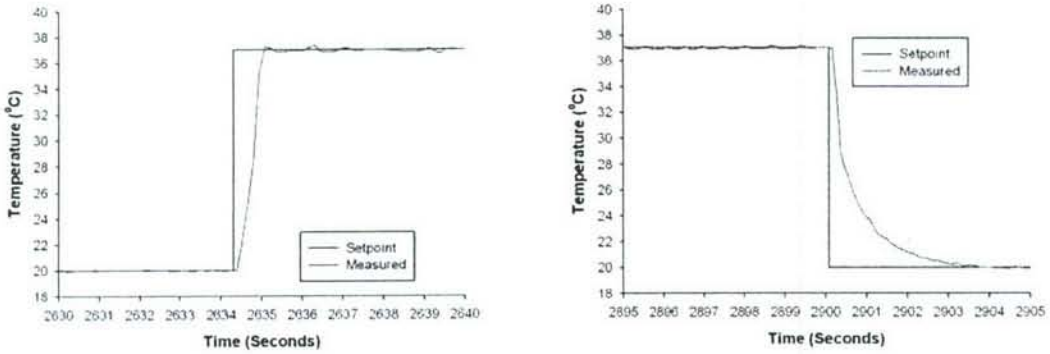


**Figure 1.** Heat-up and cool-down of the bottom surface of the cell culture dish induced by the temperature control apparatus. The response to a command step change in temperature set-point from 20°C to 37°C (left), then from 37°C to 20°C (right).

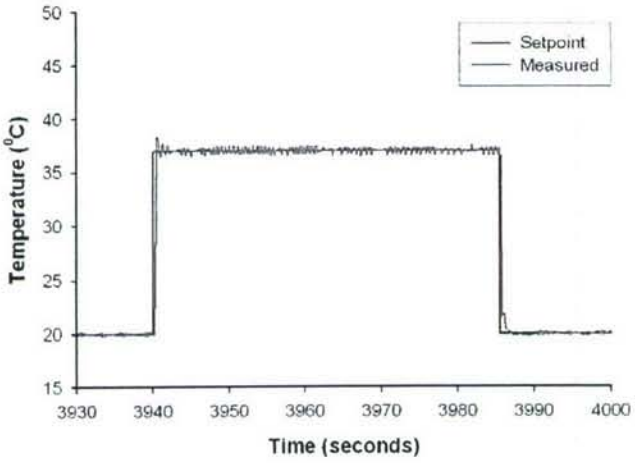


**Figure 2.** Step change response in temperature of the bottom surface of the cell culture dish coated with collagen and with chromaffin cells attached to its surface.

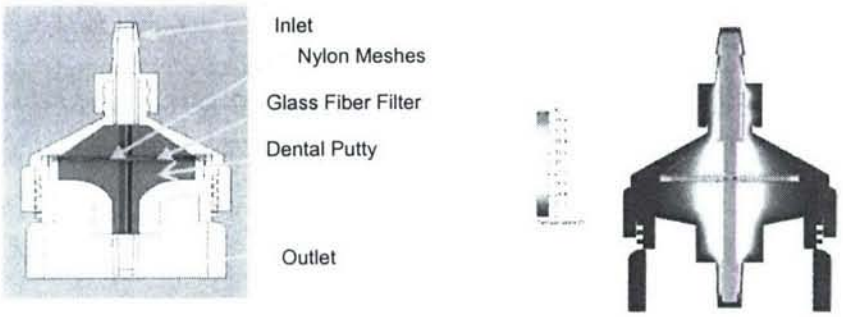


**Figure 3.** Left: CPA model cross-section; Right: Temperature distribution in the CPA without SAR.

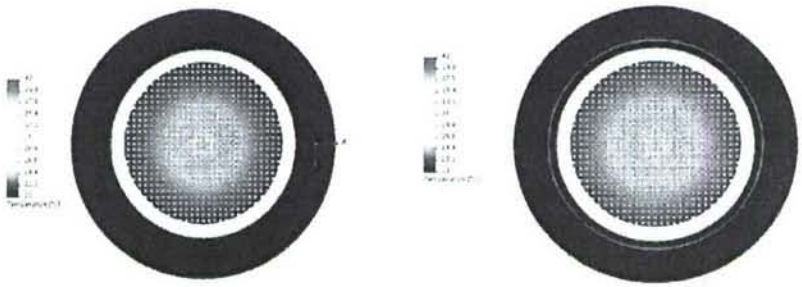

**Figure 4.** Left: Temperature distribution across the glass fiber filter without SAR; Right: Temperature distribution across the glass fiber filter with SAR modeled as a surface heat source.

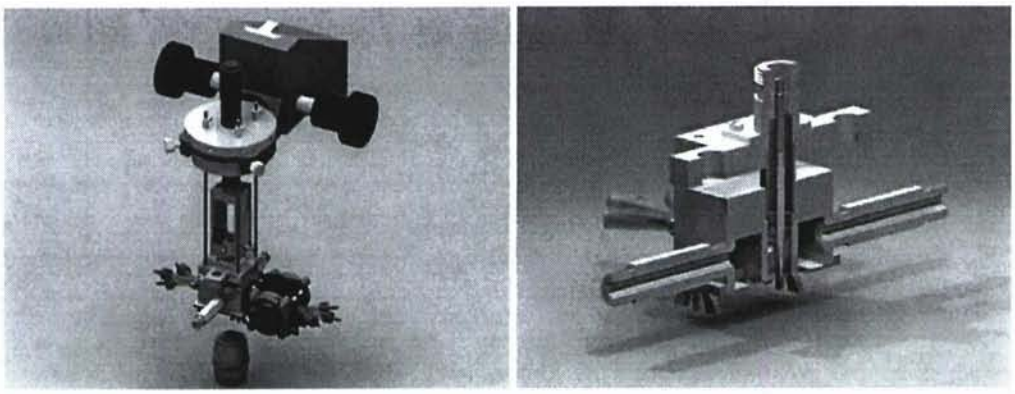

**Figure 5.** Left: Entire exposure system assembly. Right: Cross-section of exposure device.


**Figure 6.** Electric field intensity across the viewing surface of the culture dish.

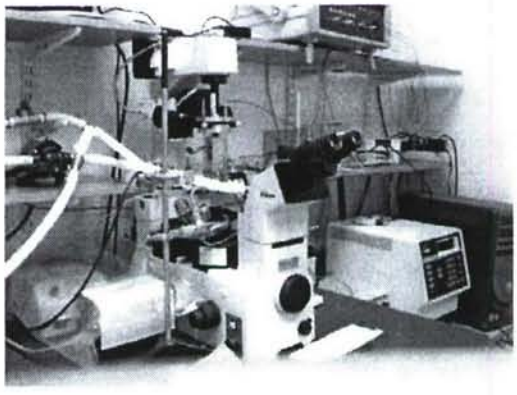

**Figure 7.** Photograph of the exposure device mounted on the inverted microscope.

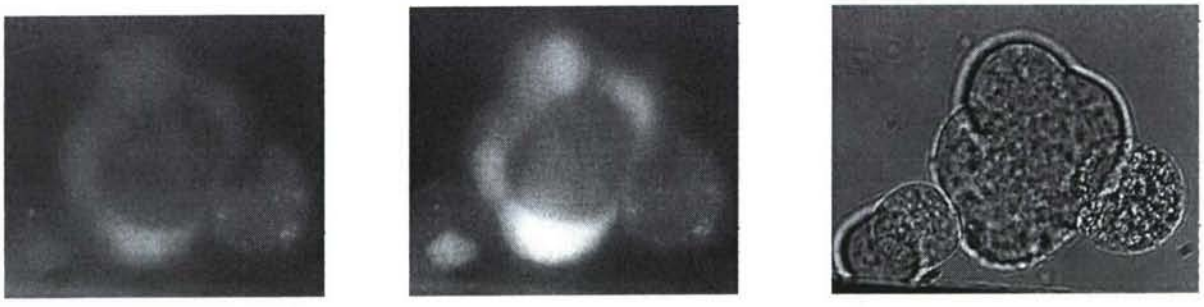

**Figure 8.** Photomicrographs of chromaffin cells loaded with Calcium-Green and exposed to a single 4 nsec, 8 MV/m pulse. Left: zero time; middle: 6 sec after the pulse; right: brightfield.